AMENDMENTS TO THE DRAWINGS

The attached sheet of drawings includes Figs. 1-3 and replaces the original sheet of drawings. The Figs. 1-3 were objected to for not being clear and legible. Applicants respectfully assert that the replacement Figs. 1-3 attached herewith are believed to be sufficiently clear and legible. Applicants also affirm that no new matter has been added by the newly submitted replacement Figs. 1-3.

Attachment: Replacement Sheet

REMARKS

Claims 1-18 are pending after entry of this paper. Claims 1-4, 9-16, and 18 have been rejected. Claims 5-8 and 17 have been withdrawn. Applicants reserve the right to pursue withdrawn and cancelled claims in a divisional or continuing application.

Claim 1 has been amended by deleting the phrase "with high sensitivity comparable or even superior to official methods." Claim 1 has been further amended changing to location of the term "in foods" to clarify that microorganisms are in general different and not just in foods. Support may be found throughout the instant specification.

Claims 11 and 13 have been amended to replace the term "bacteriosin" with the term "bacteriocin." Support may be found throughout the instant specification as filed, for example, at page 8, paragraph [0010].

No new matter has been introduced by these amendments. Reconsideration and withdrawal of the pending rejections in view of the above claim amendments and below remarks are respectfully requested.

Response to Objections made to Drawings

The drawings submitted on June 23, 2006 are objected to by the Patent Office. Specifically, the Patent Office contends that the drawings are not clear and legible. (Office Action; p. 2.)

Per request of the Patent Office, applicants respectfully submit a copy of the replacement drawings, *i.e.*, FIGs. 1, 2 and 3, which applicants believe are sufficiently clear and legible. In light of this submission, reconsideration and withdrawal of the objection to the drawings are respectfully requested.

Response to Objections made to Specification

The Patent Office contends that the use of the trademarks "Tween 20" and "UNI kit" should be capitalized and accompanied by the generic terminology. (Office Action; p. 2.)

Applicants respectfully replaced the term "Tween 20" in the specification as filed at page 8, paragraph [0010] with "Polysorbate 20 (full name Polyoxyethylene (20) sorbitan monolaurate - commercially known as TWEEN® 20)" to comply with the Patent Office's request. Any later occurrences of the term "Tween 20" were replaced with the generic terminology and the commercially known trademarked product: "Polysorbate 20 (TWEEN® 20)." Moreover, applicants also added a trademark symbol to the term "UNI®."

For the above reasons, applicants respectfully request reconsideration and withdrawal of these objections to the specification.

Response to Objections made to the Abstract

The abstract submitted with the application has been objected to because according to the Patent Office, two abstracts were submitted. The Patent Office requested clarification. Furthermore, the term "lysozyme" in one page abstract has been misspelled.

As an initial matter, applicants wish to note that since one page abstract was published by the Patent Office, applicants presumed that one page abstract was considered and reviewed by the Patent Office. Nonetheless, applicants can see how the presence of two documents entitled abstract may cause confusion. Therefore, applicants respectfully wish to advise the Patent Office, that one page abstract should be considered by the Examiner. Furthermore, to comply with the request of the Patent Office, the term "lyzocyme" was replaced

with "lysozyme." Applicants respectfully request reconsideration and withdrawal of these objections.

Response to Objections made to Claims

Claims 11 and 13 have been objected to because the term "bacteriocin" was inadvertently misspelled. Applicants respectfully submit that claims 11 and 13 have been amended to replace the term "bacteriosin" with the term "bacteriocin" per Examiner's suggestion. Reconsideration and withdrawal of the objection to the drawings are respectfully requested.

Response to Election Finality

The Patent Office argues that applicant's arguments presented in response to a restriction requirement submitted on April 24, 2009 were not found persuasive because "the species listed above do not relate to a single general inventive concept under PCT Rule 13.1," hence, "they are drawn to physically and structurally distinct products organism and SEQ ID NOS". (Office Action; p. 3.) Applicants respectfully disagree with such characterization of the invention.

As an initial matter, applicants wish to thank Examiner Shahnan-Shah for taking the time to discuss the substance of the species election on October 27, 2009. As mentioned during the discussion, even though the applicants made an election of species in order to be fully responsive to the Restriction Requirement, applicants respectfully assert the election of species is improper because it renders the instant invention unsatisfactory and inoperable for its intended purpose.

As applicants noted in the response to a restriction requirement dated April 24, 2009, the restriction requirement impedes the main feature of the present invention, *i.e.*, multiple microorganisms detection in a single operation. The invention is directed to a method of multiple microorganism detection (i.e., two or more microorganisms in foods having different properties) with a single multiplex PCR. In other words, the method provides a novel approach to detecting contaminating microorganisms including, for example, pathogenic *Escherichia coli*, *Listeria monocytogenes* and *Salmonella* species by using a single multiplex PCR reaction and plural pairs of primers. In order to detect, for example, the presence of pathogenic *Escherichia coli*, *Listeria monocytogenes* and *Salmonella* in a single operation, the claimed method would require at least three primer pairs or at least the primers disclosed in SEQ ID NOs: 1 to 6. Therefore, an election of single species and a single primer pair goes against the essential nature of the instant invention, which is to detect multiple species and **not** a single species by using a single multiplex PCR reaction and plural pairs of primers. In other words, to require applicants to elect one species and one primer pair would undermine the claimed invention and render it unsatisfactory and inoperable for its intended purpose.

Furthermore, as a side note, applicants wish to bring to the attention of the Patent Office that the International Search Report issued during the PCT phase prosecution did not raise any objections to the unity of the invention. In light of the aforementioned remarks, applicants respectfully request reconsideration and withdrawal of the finality of the restriction/election.

Response to Rejections under 35 U.S.C. §112

Claims 1-4, 9-16 and 18 have been rejected under 35 U.S.C. §112, second paragraph for indefiniteness. Specifically, the Patent Office contends that the recitation of "two

or more organisms having different properties in foods" in claim 1 presents ambiguity with respect to whether the intended microorganisms have different properties in general or only in food. (Office Action; p. 4.) Furthermore, the Patent Office alleges that the recitation of "with high sensitivity comparable or even superior to official methods" in claim 1 is not clear, especially to the reference to the "official methods." Id. Finally, the Patent Office contends that the phrase "under a culture condition" is not clear. Id. Applicants respectfully disagree.

However, in order to expedite prosecution and without disclaimer of, or prejudice to, the subject matter recited therein, applicants amended claim 1 to recite "two or more microorganisms in foods having different properties" instead of "two or more microorganisms having different properties in foods" in order to clarify that the phrase was intended to mean microorganisms having different properties in general, as made evident from the description of the same in the instant specification. Furthermore, in order to expedite prosecution and without disclaimer of, or prejudice to, the subject matter recited therein, applicants amended claim 1 to delete the phrase "with high sensitivity comparable or even superior to official methods." Finally, with respect to Patent Office's concerns about what constitutes culture conditions, applicants respectfully direct the Examiner's attention to the language of claim 2. Specifically, the culture condition is a condition in which microorganisms are allowed to grow/multiply from initial conditions of 1 CFU/100 g to 10^3 CFU/ml or more after having been cultured for 24 hours prior to the step of extracting DNA of the target microorganisms to be detected. The acronym CFU stands for Colony-Forming Unit that is a measure of viable bacterial cells. This culture condition is further described more specifically in claims 9 and 10.

Applicants respectfully request reconsideration and withdrawal of the 35 U.S.C. §112, second paragraph, rejections for indefiniteness in view of the aforementioned remarks and amendments to the claims.

Response to Rejections under 35 U.S.C. §102

Claims 1, 2, 3, 4, 11, 15, 16 and 18 have been rejected under 35 U.S.C. §102(b) as being anticipated by Aznar et al. (*Systematic and Applied Microbiology*, 25:109-119, 2002; of record). Specifically, the Patent Office alleges that Aznar teaches a method of detecting two or more microorganisms, i.e., different strains of *Listeria monocytogenes*, having different properties as made evident by Table 1 of Aznar. (Office Action; p. 5.). The Patent Office concludes that such teaching of Aznar anticipates the claimed method. Applicants respectfully disagree for the following reasons.

It is well established that "[a] claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631 (Fed. Cir. 1987). (MPEP 2131; emphasis added). In other words, in order for the Aznar reference to anticipate the claimed method of multiplex microorganism detection, Aznar must disclose each and every element as set forth in the claim either expressly or inherently. Aznar fails to do so.

First, the step of extracting DNA of the target microorganisms to be detected in the claimed method of multiplex microorganism detection is characterized in "treating [target organisms] with [(1)] a lytic enzyme and /or bacteriocin having lytic activity, [(2)] a surfactant and [(3)] a protein denaturant." Aznar et al., however, describes that the guanidium thiocyanate method of Pitcher et al. (1989) was employed in the step of extracting DNA of the target

microorganisms to be detected (page 110, right column, lines 9 - 14). This method is characterized in that guanidium thiocyanate is used as a protein-denaturing agent, but it does not teach nor suggest the step of extracting DNA of the target microorganisms to be detected with a lytic enzyme and/or a bacteriocin having a lytic activity and a surfactant as in the present invention.

Furthermore, the instant method of multiplex microorganism detection enables detection of two or more microorganisms having different properties such as *Escherichia coli* including pathogenic *E. coli* O157, and *Salmonella spp.* bacteria without being restricted to merely strains of *Listeria monocytogenes*. (Abstract, page 110, left column, lines 40 - 49, etc.).

Thus, Aznar does not teach each and every element of the claims and, therefore, as a matter of law cannot anticipate claimed invention as presented in claim 1. Applicants respectfully request reconsideration and withdrawal of the 35 U.S.C. §102(b) rejection of claims 1, 2, 3, 4, 11, 15, 16 and 18 as being anticipated by Aznar.

Claims 1, 11 and 18 have been rejected under 35 U.S.C. §102(b) as being anticipated by Brasher et al. (*Current Microbiology* 37:101-107, 1998; of record). Specifically, the Patent Office alleges that Brasher teaches a method of detecting *Salmonella*, *Vibrio*, *E. coli* and other bacteria using multiplex PCR amplification of multiple genes. (Office Action; p. 6.) The Patent Office further alleges that Brasher teaches the use of lytic enzyme proteinase K and depositing DNA by alcohol precipitation. The Patent Office concludes that such teaching of Brasher anticipates the claimed method. Applicants respectfully disagree for the following reasons.

Although Brasher describes a method of multiplex detection of microorganisms using primer pairs, the reference does not disclose each and every element as set forth in claim 1, 11 and 18 either expressly or inherently. Specifically, Brasher is silent about "treating [the target microorganisms] with (1) a lytic enzyme and /or bacteriocin having lytic activity, (2) a surfactant and (3) a protein denaturant." Each element must be present in Brasher in order to anticipate the claimed method of multiplex microorganism detection. Brasher fails to do so in at least teaching the target microorganisms with a surfactant and a protein denaturant.

In fact, Brasher teaches the use of SDS and organic solvents such as chloroform and phenol in the DNA extraction. While SDS is known as a surfactant that is easy to use in DNA extraction, it is a strong PCR inhibitor and must be removed completely after being used for extraction. Furthermore, a small contamination with SDS may also cause significant fluctuations in extraction efficiency between samples. With respect to phenol and chloroform, these are dangerous and harmful organic solvent that are not suitable for examination of pathogens in food manufacturing sites. (Specification as filed; para. [0009] page 7)

Thus, Brasher does not teach each and every element of the claims and, therefore, as a matter of law cannot anticipate claimed invention as presented in claims 1, 11 and 18. Applicants respectfully request reconsideration and withdrawal of the 35 U.S.C. §102(b) rejection of claims 1, 11 and 18 as being anticipated by Brasher.

Response to Rejections under 35 U.S.C. §103

Claims 1-4, 9-16 and 18 have been rejected under 35 U.S.C. §103(a) as being unpatentable over Aznar et al. (*Systematic and Applied Microbiology*, 25:109-119, 2002; of record). Specifically, further to the discussion of anticipation of the claimed invention by Aznar

addressed above, the Patent Office concedes that Aznar does not teach certain limitations such as pH, medium components or chemicals used to lyse the microorganism before DNA extraction. (Office Action; p. 7). However, the Patent Office alleges that these limitations are experimental parameters and it would have been *prima facie* obvious to a skilled artisan to develop such conditions. "[O]ne of ordinary skill in the art would have been motivated to by teaching of Aznar et al. to optimize the culture media and pH to obtain the best results for a Multiplex PCR assay." Id. Applicant respectfully disagree with such reasoning and conclusion arrived by the Patent Office.

Applicants respectfully assert that the instant invention is not anticipated, nor is it made obvious by Aznar. Applicants respectfully assert that the Patent Office improperly applied "obvious to try" rationale in support of an obviousness rejection.

> An "obvious to try" rationale may support a conclusion that a claim would have been obvious where one skilled in the art is choosing from a finite number of identified, predictable solutions, with a reasonable expectation of success. (MPEP 2145; emphasis added)
>
> [However, it would be improper to find obviousness where] what would have been 'obvious to try' would have been to vary all parameters or try each of numerous possible choices until one possibly arrived at a successful result, where the prior art gave either no indication of which parameters were critical or no direction as to which of many possible choices is likely to be successful. *Id.*

Therefore, Aznar does not make the claimed invention *prima facie* obvious because Aznar gives no indication of which parameters were critical or no direction as to which of many possible choices is likely to be successful. For instance, (1) Aznar describes the detection of multiple *Listeria monocytogenes* strains. Aznar does not describe the detection of two or more microorganisms that have different properties such as *E. coli* O157 and *Salmonella*

spp. in addition to *Listeria monocytogenes*. (2) Aznar employs the Pitcher method of extracting the DNA with guanidium thiocyanate. Aznar does not teach nor suggest "a step of extracting DNA of the target microorganisms to be detected, by using a lytic enzyme and /or bacteriocin having lytic activity, or a surfactant, other than a protein denaturant." For example, *Listeria* is Gram positive bacteria (a thicker and higher density peptide glycan layer), whereas *Salmonella* and *E.coli* are Gram negative bacteria. Thus, the DNA extraction methods that work for one species, does not necessarily would work for the other, and a skilled artisan would have to conduct a great deal of undue experimentation in order to arrive at the claimed invention based on the teaching of Aznar. In fact, since Aznar only utilizes *Listeria moncytogenes*, a skilled artisan would not look beyond the Pitcher method of extracting the DNA with guanidium thiocyanate. (3) It is relatively easy to proliferate bacteria simultaneously, when the target microorganisms, as in Aznar, belongs to the same family or bacterial species of same genus, but it is difficult to do so, and would require undue experimentation, for microorganisms of diverse types such as *E. coli*, *Salmonella spp.* and *Listeria monocytogenes*. For example, *Listeria* grows at a low temperature and proliferates slower compared to *Salmonella spp.* and *E. coli O157*. (4) It is shown in Aznar that annealing temperature applied for 9 types of primers used in the *Listeria monocytogenes* detection varies over as much as 6 levels ranging from 48°C to 65°C (see Table 2). Under such conditions, the detection would need to be carried out by changing various parameters in PCR for each primer even when the detection target is only *Listeria monocytogenes*. On the other hand, the technical substance of a detection method of plural bacteria using PCR as the method of the instant invention lies in the provision of a PCR system in which a series of steps using primer pairs comprising a plurality of nucleotide sequences is conducted in a single procedure without the need of settling plural conditions. This aspect is

technically distinct from the method of Aznar in which multiple *Listeria monocytogenes* strains shown in Table 1 were detected by any one of the primers with only few exceptionally non-detected cases.

In view of the aforementioned remarks, applicants respectfully asset that the instant invention is not made obvious by Aznar. Applicants respectfully assert that a skilled artisan lacks a finite number of identified, predictable solutions, with a reasonable expectation of success to support the "obvious to try" rationale put forward by the Patent Office. Therefore, applicants respectfully request reconsideration and withdrawal of the 35 U.S.C. §103(a) rejection of claims 1-4, 9-16 and 18 as being obvious over Aznar.

Dependent Claims

The applicants have not independently addressed all of the rejections of the dependent claims. The applicants submit that for at least similar reasons as to why independent claim(s) 1 from which all of the dependent claims 2-4, 9-16 and 18 depend are believed allowable as discussed *supra*, the dependent claims are also allowable. The applicants however, reserve the right to address any individual rejections of the dependent claims and present independent bases for allowance for the dependent claims should such be necessary or appropriate.

Thus, applicants respectfully submit that the invention as recited in the claims as presented herein is allowable over the art of record, and respectfully request that the respective rejections be withdrawn.

CONCLUSION

Based on the foregoing amendments and remarks, the applicant respectfully requests reconsideration and withdrawal of the election requirement of claims and allowance of this application.

AUTHORIZATION

The Commissioner is hereby authorized to charge any additional fees which may be required for consideration of this Amendment to Deposit Account No. **50-4827**, Order No. 1004451.001US.

In the event that an extension of time is required, or which may be required in addition to that requested in a petition for an extension of time, the Commissioner is requested to grant a petition for that extension of time which is required to make this response timely and is hereby authorized to charge any fee for such an extension of time or credit any overpayment for an extension of time to Deposit Account No. **50-4827**, Order No. 1004451.001US.

Respectfully submitted,
Locke Lord Bissell & Liddell LLP

Dated: October 29, 2009

By: /Serge Ilin-Schneider/
Serge Ilin-Schneider, Ph.D.
Registration No. 61,584

Correspondence Address:
Locke Lord Bissell & Liddell LLP
3 World Financial Center
New York, NY 10281-2101
(212) 415-8600 Telephone
(212) 303-2754 Facsimile